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Review

Changing times: DNA resequencing and the "nearly normal autopsy"

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Abstract

No matter how meticulous the autopsy, non-traumatic deaths in the young go unexplained from 5–10% of the time. The percentage is higher in children and young adults. Advances in molecular biology and DNA technology now make it possible to explain many of those deaths. This development is not without irony. At the same time that many clinicians are expressing frustration about the lack of tangible gains provided by the Human Genome Project [Greenhalgh T. The Human Genome Project. *J R Soc Med. Dec* 2005;**98**(12):545], and pathologists are wondering about the viability of their field, DNA technology is about to reshape the field of forensic pathology. Emerging evidence suggests that the underlying cause of death in many is genetic, and that both the heart and liver abnormalities can both play a role. The problem is that death from a wide variety of genetic defects may leave no histological markers. The ability to identify these "invisible diseases" with postmortem genetic testing has become a reality far more quickly than anyone had ever imagined. The US Food and Drug Administration is about to place "black box" warnings on warfarin advising doctors screen potential recipients for the ability to metabolize that drug and the American Heart Association has recently editorialized that because of genetic-induced variations in electrical conduction that all newborns should have a screening electrocardiogram before they leave the hospital. The introduction of large-scale genetic screening will have an enormous effect on the practice of forensic pathology, far beyond anything seen in our lifetimes. It will also change the practice of medicine as we know it. This paper reviews the current status of the problem.

Keywords: Sudden cardiac death; Normal autopsy; Hypertrophic cardiomyopathy; LQTS; P450 polymorphism; Drug death; DNA array; Ryanodine

1. How big is the problem?

1.1. The medical examiner's experience

The information in Table 1 was derived from a study of 432 consecutive sudden cardiac death (SCD) victims aged 31–40 years, investigated by the State of Maryland Medical Examiner's Office.[2] The most common cause of death was arteriosclerotic heart disease (ASHD), accounting for 60% of all cases. The second most common diagnosis was "undetermined," accounting for 9%. The experience in other US jurisdictions is more or less similar.

A study of 126 cases of non-traumatic sudden deaths among military recruits, published in 2004, found that the most common cause of sudden death was an identifiable cardiac abnormality, present in 64 of 126 recruits (51%). The cardiac abnormalities mainly involved the coronary arteries (39 of 64 recruits or 61%), with myocarditis accounting for 20% of cases, and hypertrophic cardiomyopathy for 13%. After a thorough autopsy and complete toxicology screening, the cause of death remained unexplained in slightly more than a third of the cases.[3]

In another recent autopsy study, a decade-long review in Sydney involving only individuals aged 5–35 years, heart disease accounted for nearly 60% of deaths, and the most common type of heart disease, found in 29% of the cardiac cases, was SCD in individuals with normal, or nearly normal hearts.[4] The usual suspects made up the rest of the cases: acute myocardial infarction (24.5%), myocarditis (11.6%), hypertrophic cardiomyopathy (5.8%), aortic dissection (5.4%), and dilated cardiomyopathy (5.4%). Overall, this group found that 4.3% of deaths in this age group went unexplained.

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Table 1
Data adopted from Virmani et al., Ref. [2]

Causes of death, ages 31–40	
Atherosclerosis	60%
No finding	9%
Hypertensive left ventricular hypertrophy	6%
Idiopathic left ventricular hypertrophy	4%
Dilated cardiomyopathy	4%
Myocarditis	3%

1.2. Postmortem DNA studies

Many "nearly normal hearts" are, in fact, genetically abnormal, but it is difficult to calculate just how many. In 2004, Chugh and colleagues estimated the prevalence of mutations in SCD arrhythmia-related candidate genes. The group performed genetic analysis on tissue blocks from 12 of 270 SCD victims previously found to have anatomically normal hearts. This group of cases had been accumulated over a 13-year period. Archived, paraffin-embedded myocardial tissue blocks of various ages were examined for KCNQ1 (KVLQT1), KCNH2 (HERG), SCN5A, KCNE1, and KCNE2 mutations. Two of the 12 SCD victims were found to have a defect in the HERG gene.[5] The real incidence of HERG mutations would have almost certainly been much higher than Chugh reported. DNA can be extracted from paraffin blocks, but formalin fixation damages DNA, and even in the absence of formalin, DNA degrades over time. Similar problems with DNA extraction may explain why Lunetta found an even lower incidence when attempting to determine the prevalence of long QT syndrome (LQTS) mutations in cases of drowning. Retrospective screening for specific founder mutations in KCNQ1 (KVLQT1) and KCNH2 (HERG) genes in 165 consecutive individuals who had drowned in Finnish waters disclosed a single KCNH2-Fin mutation in a 44-year-old woman whose death had initially been classified as the result of suicidal drowning.[6]

Genetic screening of SIDS victims confirms that this group of disorders has a fairly high incidence. Amestad and his colleagues performed genetic screening in 201 Norwegian SIDS victims. Mutations of rare genetic variants were identified in 10% of cases.[7] Roughly half the mutations involved variants of SCN5A gene.[8] On further analysis, there was evidence that the children suffered from defects in the voltage-dependent inactivation of sodium currents, leading to long QT syndrome. The editors of Circulation, where the papers were published, concluded that this disease is sufficiently common to warrant the EKG screening of all newborn children,[9] not an inconsiderable undertaking. If a disease is sufficiently common to require newborn screening, then it should certainly be considered as a possible cause of death. Fortunately, techniques are now available by which long QT syndromes can be ruled out by analysis of postmortem materials. At present these techniques are very expensive – $5000–6000 per case when done by commercial laboratories.

1.3. Clinical estimates of genetic disease

1.3.1. Retrospective population studies

Krahn et al. examined individuals who had survived an out-of-hospital cardiac arrest, but who were found to have no evidence of cardiac disease (normal left ventricular function, normal coronary arteries, and normal resting corrected QT). He infused them with adrenaline and procainamide in an effort to unmask any underlying electrical disorders. In addition, family members also underwent non-invasive screening and provocation testing with the same drugs. Eighteen patients (mean ± SD age, 41 ± 17 years; 11 female) with unexplained SCD were studied. The final diagnosis was catecholaminergic polymorphic ventricular tachycardia (CPVT) in 10 patients (56%), Brugada syndrome in two patients (11%), and unexplained (idiopathic ventricular fibrillation) in six patients (33%). Of the 55 family members (mean ± SD age, 27 ± 17 years; 33 female), nine additional individuals were detected from two families; there was a single case of Brugada syndrome and eight patients with CPVT. Compared to the number of people who suffer from ASHD, these numbers are small. On the other hand, the numbers are much higher than anyone would have predicted several years ago, and it appears that genetically-linked arrhythmic disorders are not nearly so uncommon as was once thought.[10]

1.3.2. Prospective population studies

The Pavia project was designed to collect EKG records and demographic information from 50,000 neonates between the 15th and 25th day of life.[11] Whenever a child with a QT interval greater than or equal to 470 ms was identified, their DNA is sequenced. As of November 2005, 33,152 neonates had been enrolled. The corrected QT interval exceeded 440 ms (which is abnormal, but not diagnostic for LQTS) in 477 infants (1.4%), and exceeded 470 ms (diagnostic for LQTS) in 31 infants, giving prevalence in infants of nearly 1 in 1000; nearly 50% of these children with prolonged QT intervals are thought to be carrying a long QT mutation.

Molecular sequencing has been completed in half of the 31 infants with a corrected QT interval exceeding 470 ms. LQT mutations have been identified in half of those (5/8 or 62% had KCNQ1, 2/8 or 25% had KCNQ21, and 1/8 or 12.5% had SCN5A mutations). But, as with archival tissue, the actually numbers are likely to be much higher, though for somewhat different reasons. Firstly, even among patients who obviously have LQTS, sequencing identifies mutations only 65–70% of the time. Secondly, many of the children with corrected QT intervals >440 ms, but still less than 470 ms, are also likely to be carrying mutations.

2. The microarray and the genetic revolution

Until recently Brugada syndrome, recognized less than 15 years ago,[12] and hypertrophic cardiomyopathy (HCM),

first described in 1958,[13] generally received little notice in the forensic community, and not a great deal more attention among clinicians. Surgery can be done to enlarge the outflow tract of those affected with florid HCM, but until the advent of the internal defibrillator, there was little that could be done to prevent death from lethal arrhythmia once a carrier had been identified. Yet, within a year or so, it will be possible to analyze ''nearly normal'' hearts for virtually every genetic abnormality conceivable, including HCM. How does the technology work?

Oligonucleotide or cDNA microarrays (also called gene or DNA chips) are now produced using a variety of technologies. The most common type of microarray measures the mRNAs transcribed from genes that encode proteins. RNA is extracted from a cell, converted to complimentary DNA (cDNA) or complimentary RNA (cRNA) that is then amplified by PCR. Fluorescent molecules, also called tags, are incorporated into the final product.

If a cDNA or cRNA molecule contains a sequence complimentary to one of the single-stranded probe sequences incorporated in the array (called reporters, and thousands of reporters may be attached to one chip), it will hybridize to the spot where the complementary reporters are fixed. The result is the emission of fluorescent light that can be detected by an automated scanner. The intensity of the fluorescence measured is roughly proportional to the number of copies of a particular mRNA that are present, providing a semi-quantitative guide to the activity of the gene that has been identified. It is now possible to affix reporters for all of the genes capable of causing hypertrophic cardiomyopathy (HCM) to one chip and screen for all 150 + HCM mutations at one time, over the course of a few days.[14] Screening for P450 enzyme abnormalities is even simpler, and test devices are already being sold commercially (Roche-Affamatrix™ system). The test can be performed on postmortem liver tissue in a matter of hours.[15]

3. Genetically-linked heart disease

Table 2 contains a list of the principle types of heart disease that can be diagnosed with DNA testing. Readers may be surprised by the inclusion of viral myocarditis, but recent studies have shown that viral genomes may be present in cardiac tissue, even if histological evidence of infection is not (see below). A truly inclusive list of ''DNA diseases'' would also include the mutations responsible for abnormal lipid metabolism and coronary artery disease (CAD), or even atrial fibrillation. But, forensic pathologists have no difficulty recognizing coronary artery disease and a history of atrial fibrillation is a clinical finding confined to the living, so those problems will not be considered here.

Hypertrophic cardiomyopathy is the most common mutation causing SCD. It is an autosomal dominant affecting 1 in 500 people (or about 0.2% of the general population). Only a small fraction of those with the genotype ever develop clinical symptoms, but death is particularly likely in competitive athletes because one manifestation of their disease is normal or supernormal ventricular function; ejection fractions of 65–80%, allow affected individuals to perform as superior athletes competing at local, national, or even international levels. Investigation of these deaths places particular pressure on the medical examiner and his team.[16]

Table 2
Recognized causes of sudden death not associated with anatomic abnormality

Hypertrophic cardiomyopathy
Long QT syndromes
LQT1 (KCNQ1)
LQT2 (KCNH2)
LQT3 (SCN5A)
LQT4 (ANK2)
LQT5 (KCNE1 also called MinK)
LQT6 (KCNE2 also called MirPI)
LQT7 (KCNJ2)
LQT8 (CAV1.2 – a calcium channel)
Drug-HERG interactions
Brugada syndrome (SCN5A)
Catecholaminergic polymorphic ventricular tachycardia (RyR2)
Idiopathic ventricular fibrillation (SCN5A)
Progressive cardiac conduction disease (SCN5A)
Short QT syndrome (KCNQ1 and KCNH2)
Myocarditis (DNA genome may be present even in absence of infiltrate)
Hepatic P450 mutations

Cardiac entities are listed in probable order of frequency, though there are no estimates for the frequency of Dallas criteria negative myocarditis.

HCM mutations have been identified in 14 different genes. Most are single nucleotide substitutions found within coding exons, intron–exon junctions, or promoter regions of the genes for β-myosin heavy chain, cardiac troponin T, or myosin binding protein-C. Clusters of mutations have been described in the β-myosin heavy chain gene (*MYH7*) that are generally associated with marked hypertrophy and poor prognosis.[17]

In most, but not nearly all cases of HCM, there is some degree of left ventricular hypertrophy, but several different patterns of HCM are recognized. The most common pattern, seen in approximately 2/3 of the cases, involves asymmetric hypertrophy of the inter-ventricular septum. However, other patterns are possible. In approximately 25%, the entire ventricle is involved, and in a small proportion of patients (approximately 10%), hypertrophy is confined to the apex. This pattern is much more common in Japan than in the West.[18] Traditionally, it has been presumed that when sudden death occurred in patients with HCM, it was, somehow, a consequence of myocyte disarray and fibrosis (see Fig. 1). It is now clear that adrenergic stress of any sort can produce the same type of disarray,[19] and that the presence of disarray is far from diagnostic for any one disorder. A strong association between hypertrophy and arrhythmia exists, but the mechanism by which myocyte enlargement increases arrhythmic risk may have more to do with changes in intrinsic automaticity of the

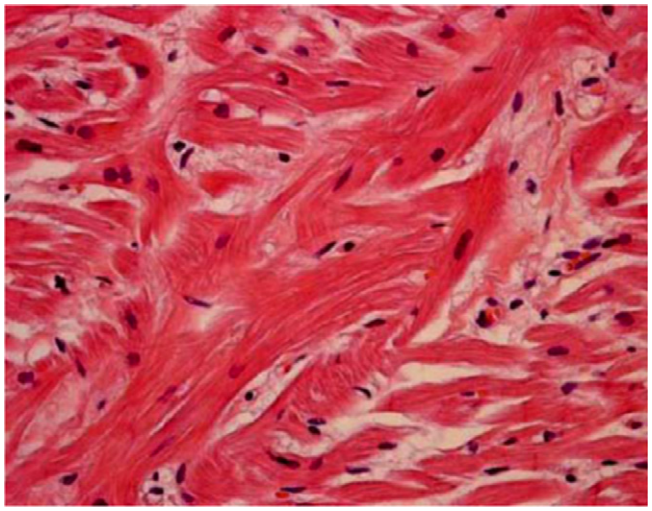

Fig. 1. An example of myocyte disarray. It had been thought that this lesion was diagnostic for hypertrophic cardiomyopathy. More recent studies have shown that disarray can be seen whenever there is adrenergic stress. From Ref. [16], reprinted with permission.

heart itself, rather than with the size or shape of the individual myocytes.

The problem for medical examiners is that one of the HCM mutations, involving alpha-tropomyosin, results in little, if any, hypertrophy, but is associated with a high incidence of SCD.[20] Thus, the medical examiner's most dreaded case: the high-profile death of a prominent young athlete with no apparent cause, and no explanation in sight. Now that a prototype chip, capable of detecting mutations in HCM genes has already been developed,[14] that situation should change shortly Fig. 2.

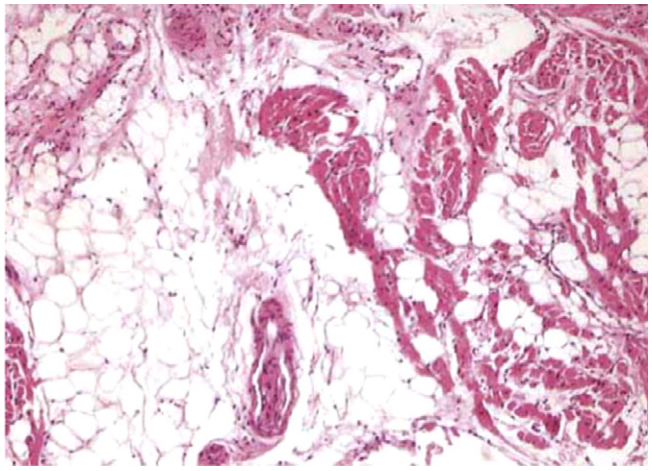

Fig. 2. Fatty replacement of the right ventricle is a known cause of sudden cardiac death due to mutation in the ryanodine gene. RyR1 mutation in skeletal muscle causes malignant hyperthermia and central core disease. In cardiac muscle, RYR2 mutations lead to catecholaminergic ventricular tachycardia (CPVT), but other mutations in the same gene can lead to the occurrence of right ventricular dysplasia. Reproduced, with permission, from Ref. [33].

3.1. LQTS syndromes

The QT interval, that portion of the electrocardiogram measured from the beginning of the QRS complex to the end of the T wave,[21] represents the total duration of ventricular systole. It should be less then half as long as the time elapsed from one "r" wave to the next. If it is longer, then the process of repolarization will be prolonged and the chances of an "r" wave falling on a "T" wave (and triggering a fatal arrhythmia) are enhanced. Put another way, the shorter the r–r interval, the higher the heart rate, and the more likely it is that ventricular activation will occur during the vulnerable T wave. This explains why more than 80% of LQT1, 2, 3 deaths occurring during exercise or after being startled.

One in 5000 people carry these defects, but if acquired forms of LQTS are included in the estimate, the true incidence of LQTS may be closer to 1 in 1000.[22] This group of syndromes involves at least eight different gene, mutations, any one of which leads to the production of malfunctioning ion channels, though some of these mutations are very rare (disorders such as idiopathic ventricular fibrillation,[23] progressive cardiac conduction disease,[24] and the only recently recognized short QT syndrome.[25]) The defects can be lumped into two groups; those involving either the alpha or beta channel subunits. Think of a peeled orange that has been separated into segments. If the segments are put back together, the result will look more or less like an orange, but only if a string or rubber band is placed around the circumference, in order to hold the segments upright, and if any of the segments contain too many seeds, the segment itself is not very edible and may even look different than the other segments (i.e., it is non-functional). Think of each segment as one subunit of an ion channel and the string holding them together as one of the assembler proteins. Changes in any of the "segments" can cause sudden death, even though they are completely invisible to the naked eye Fig. 3.

The nomenclature for these disorders is confusing. Genes that encode the potassium channels *KVLQT1* (chromosome 11) and *minK* (on chromosome 21) interact to form the cardiac I_{Ks}, the channel that controls the slow inward potassium current. If the mutation involves KVLQT1, the resulting syndrome is called LQT1; if it is in *min*K the syndrome is called LQT5.[26]

The HERG potassium channel gene (on chromosome 7) and the *MiRP1* gene (on chromosome 21) interact to form I_{rk}, the inward rapid potassium current. If there is a defect in HERG, the resultant syndrome is called LQT2; if on *Mir* it is called LQT6. LQT3 is due to mutations in the sodium cardiac channel SCN5A, and the resulting syndrome is commonly referred to as Brugada syndrome. The LQT4 syndrome is due to a mutation in the ankyrin-B protein.[27] LQT7 is the result of yet another defect in the alpha-subunit of the I_{Kr} channel. No matter the defect, the result is the same: prolonged repolarization. Prototype microarrays capable of identifying essentially all of the LQTS syn-

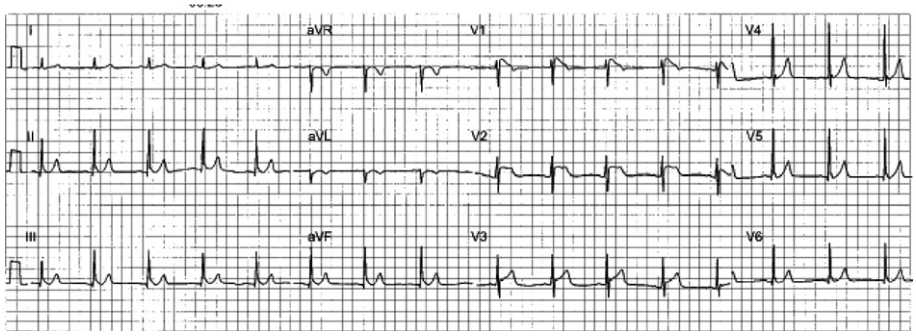

Fig. 3. Typical Brugda syndrome tracing with prominent J-point elevation, followed by downsloping ST-segment elevation in V1, horizontal ST-segment in V2, upsloping ST-segment elevation and positive T wave in V3 and normal V4. Reproduced with permission from Gussak and Antzelevitch, Journal of Electrocardiology, 2000.

dromes have already been built.[28] The process is still expensive and labor intensive – $US 5400 for comprehensive testing of the first six long QT genes in the decedent, and the process may take up to six weeks.[29]

As a practical matter, the QT interval can be prolonged by blockade of any of the different potassium channels, but most, if not all, clinical cases of drug-induced Torsades des pointes (TdP), a lethal form of ventricular tachycardia, are due to abnormalities of the (HERG) ion channel, the one that conducts the rapid component of the delayed rectifier KC current I_{Kr}.[30]

LQT3 is a much rarer syndrome (less than 5% of LQTS), the result of a "gain of function," mutation producing Na channels that remain open, or "loss of function," where Na channels stay closed (which is the defect in Brugada syndrome). When the channel remains open, it is responsible for the death of young Asian men who die during nightmares (the disorder known as *bangungut*). The fascinating aspect of the mutation is that it can be associated with morphological changes, even if only at the microscopic level. Biopsies of 18 patients believed to have this syndrome (based on symptoms and typical electrocardiographic symptoms), contained detectable SCN5A mutations in only four patients, but structural alterations were seen in 14: right ventricular micro aneurysms in seven patients, and four of these also had micro aneurysms on the left. Localized right ventricular myocarditis in 14 patients, and viral genomes were found in four; right ventricular cardiomyopathy was present in one patient; and cardiomyopathic changes were noted in three additional cases.[31] The findings reinforce the relatively recent notion that there may also be acquired forms of Brugada syndrome.[32]

3.2. The ryanodine receptor

The ryanodyine receptor (Ry) is the single largest gene in the human body, with 102 exons. Mutations are associated with SCD, even though the heart appears structurally normal, and the QT interval is not prolonged. Death occurs as a result of stress-induced bidirectional ventricular tachycardia, also called catecholaminergic polymorphic ventricular tachycardia (CPVT). These episodes occur because of abnormal calcium concentrations within the individual myocytes.

Experiments with cloned Ry receptors have shown that mutations make the receptor inherently unstable and prone to unpredictable failure, allowing the cell to be flooded with too much calcium at the wrong point in the contraction cycle, resulting in electrical instability.[33]

Three different genes encode three different iso-forms of the ryanodine receptor. Ry1 and Ry2 are found mainly in muscle (Ry2 heart), but also other types of cells, even lymphocytes. Ry3 is ubiquitous, but its function is unknown. At least 10 different mutations have been identified. Ry mutation in skeletal muscle causes malignant hyperthermia (anesthesia deaths) and central core disease. In cardiac muscle, RYR2 mutations lead to CPVT, but other mutations in the same gene can lead to the occurrence of right ventricular dysplasia.[34,33]

3.3. Myocarditis

Based on autopsy studies which, for the moment, are the only way to estimate prevalence, the incidence of myocarditis in the industrialized world is on the order of 10%, and perhaps even higher in those under age 35.[35,36,4]

Prevalence varies from country to country (in parts of South America, Chiagistic myocarditis is common, but it is unheard of in Europe), and it is not at all rare to discover clear-cut evidence of myocarditis in asymptomatic individuals who have died of trauma. New, non-invasive modalities for the diagnosis of myocarditis, such as contrast MRI,[37] have recently become available, but they are unlikely to be of much value after death. Definitive diagnosis of myocarditis still requires the demonstration of inflammatory infiltrates in the myocardium, and that can only be done with biopsy or on the autopsy table. Even then, it is becoming increasingly obvious that pure histological criteria are inadequate for diagnostic purposes, and that some decedents with normal appearing hearts have actually died of an occult viral infection.

In 2003, researchers studied 24 consecutive patients admitted within 24 h after onset of chest pain. Each patient manifested all the symptoms of acute coronary syndrome

with electrocardiographic changes, elevated levels of troponin T, and elevated CPK. When angiography was negative, biopsies were obtained, divided in half, and then analyzed both histologically, and with DNA testing. DNA from each biopsy sample was analyzed (nested polymerase chain reaction/reverse transcriptase–polymerase chain reaction) for all of the viruses most commonly associated with myocarditis (EV, ADV, PVB19, human cytomegalovirus, Epstein–Barr virus, *Chlamydia pneumoniae*, influenza virus A and B, and *Borrelia burgdorferi* genomes). In addition, both acute and convalescent serum samples were collected for later measurement of viral titers.

Quite surprisingly, only one patient met the standard histological criteria (Dallas) for myocarditis. But PVB19, EV, and ADV genomes were detected in the myocardium of 12, three, and two patients, respectively (71% of the group). Follow-up biopsies of virus-positive patients (11 of 17) demonstrated persistence of PVB19 genomes in 6 of 6 patients, EV genomes in 2 of 3 patients, and ADV genomes in 1 of 2 patients, respectively. In sum, viral genomes can be demonstrated in 71% of patients with normal coronary anatomy, clinically mimicking acute myocardial infarction, presumably from coronary artery spousm, and Parvo B-19 is the agent most frequently responsible.[38,39]

Others have observed similar findings. Kyto et al. reexamined the cardiac histopathology in 142 cases where death had previously been attributed to myocarditis.[38] Three experienced pathologists using the Dallas criteria reviewed the slides independently. Only 32% of the 142 subjects met the Dallas criteria for myocarditis (75% of pediatric and 28% of adult patients, $P = .001$).

4. Drug deaths

Long-term drug abuse produces anatomic and neurochemical changes that favor SCD, particularly among stimulant abusers. Many of these changes are plainly visible in the heart: microfocal fibrosis, myocyte hypertrophy, and hypertrophy of the media of the intramyocardial resistance vessels.[40,41] However, many other changes are invisible, because they involve genetic defects.

Cocaine, for example, binds to the HERG channel and, therefore, has the potential of causing QT interval prolongation and, perhaps, a lethal episode of (TdP).[42] This ability has nothing to do with visible morphologic changes produced by long-term cocaine use. Rather, it is the result of the blockade of potassium channels responsible for terminating the plateau phase of the action potential. Although there are many candidate channels, blockade of HERG, which controls the rapid component of the delayed rectifier current, appears to be responsible for most, if not all, clinical cases of drug-induced TdP, no matter the drug.

The reason that more people with HERG mutations do not die of TdP after taking drugs known to interact with the HERG channel is that HERG blockade is only one of many factors that act together (the ''multiple hit'' theory) to cause prolonged ventricular repolarization. These other factors include low serum potassium concentrations, slow heart rate, other genetic factors, and the co-administration of other drugs that either block HERG channels or interfere with drug metabolism.[30] Circumstances must be just right, and the decedent very unlucky.

In practice, a pathologist may detect minor structural variants, such as minimal perivascular fibrosis, some interstitial fibrosis, or perhaps medial hypertrophy in some small vessels, but no one obvious lesion, or set of lesions that could account for death. If that same pathologist were able to determine whether or not a HERG mutation was present, it would likely have a considerable effect on their final diagnosis.

5. Genetic forms of liver disease

The activity of P450 cytochrome system is genetically determined and varies from person to person, and race to race. Depending on which enzyme contains the mutation, and how much the mutation alters enzyme activity, individuals are classified as: ultra-rapid, extensive, intermediate, or poor metabolizers. Depending on the drug metabolized, toxicity may be entirely a function of metabolizer status.

For example, a depressed patient might not experience the anticipated benefits of drug therapy. The antidepressant amitriptyline is actually a pro-drug, and it only becomes active when it is oxidized to form nortriptyline. The conversion is accomplished by the P450 CYP2D6 enzyme system. A ''slow metabolizer'' taking the recommended dose of amitryptiline would not experience any therapeutic benefit because they would be unable to convert enough amitriptyline into nortriptyline. On the other hand, an ''extensive metabolizer,'' taking the same dose, might become toxic because they produce too much active drug.

The clinical significance of these observations is very great, but the forensic impact may be even greater. In a case that recently made its way through the US Courts system, a nine-year-old with multiple developmental disorders was admitted to hospital with seizures. He was under a pediatrician's care, and was receiving methylphenidate, clonidine, and fluoxetine. The seizures proved uncontrollable and the child died after several days of treatment. Postmortem blood testing disclosed very high levels of fluoxetine, but no other apparent cause of death. State authorities immediately entered the picture, concerned that the parents were incompetent, or had actually murdered the child. Several other children were removed from the household. Samples of the decedent's liver were tested using a DNA array system. It was determined that the child had no functional CYP2D6, and could not form the main metabolite of fluoxetene, causing concentrations of that drug to reach toxic levels (fluoxetine is demethylated by CYP 3A4/5 and possibly by 2C9/19). Both fluoxetine and norfluoxetine are hydroxylated by CYP 2D6. The accumulation of fluoxetine might have been due to the boy's lack of CYP 2D6 enzyme for hydroxylation. The investigation was dropped by

police, but almost certainly would not have been if the P450 status had not been evaluated.[43]

The death of this child illustrates the problems of prescribing when the CYP2D6 and CYP2C19 metabolizer status of depressed patients is not known. Preliminary average dose suggestions based on the phenotype or genotype have existed since 1991. This is a first attempt to apply the new pharmacogenetics to suggest dose-regimens that take the differences in drug metabolic capacity into account.[44] These recommendations are equally appropriate for clinicians and forensic scientist alike. The need for near-instant screening is immediate and real.

During the first week of 2006, researchers studying the interaction between the serotonin 1B receptor [5-hydroxytryptamine (5-HT1B) receptor] which interacts with brain protein p11, found that increasing concentrations of p11 brain protein caused increased amounts of 5-HT1B to localize at the cell surface. Concentrations of p11 are increased in rodent brains by antidepressants or electroconvulsive therapy, but decreased in animal models of depression. They are also decreased in brain tissue taken from depressed patients. Over expression of p11 increases 5-HT1B receptor function in cells and recapitulates certain behaviors seen after antidepressant treatment in mice. And, when knockout mice are engineered so as not to make p11, they exhibit a depression-like phenotype, reduced responsiveness to 5-HT1B receptor agonists and reduced behavioral reactions to an antidepressant.[45] At this rate it is quite conceivable that psychiatrists may be implementing array technology even before the forensics community.

6. Conclusions

If microarray devices were only intended for postmortem or forensic investigations, it would be years before the devices became available. The market is simply too small to be financially interesting. However, clinical demand will be enormous, and that demand will explode much sooner than anyone thinks. Reagent makers will be forced to produce ever cheaper, increasingly sensitive devices very quickly and that means there will be little time to sort out rules for who should be tested, and by what means. If one entertains any doubts about the immediacy of these developments, one need look no further than the administrators of the US Food and Drug Administration, which is about to alter or add another "black box" warning to warfarin. The label will contain a strong warning that patients being treated with warfarin be genetically screened before beginning therapy. Like codeine, warfarin is metabolized by CYP2D6, and 3–20% of American are genetically deficient – i.e., they cannot break down the drug down, and should receive lower doses. The FDA is even more concerned about testing for a subunit of vitamin K called the epoxide reductase subunit (abbreviated as VKORC1) which is warfarin's target. More than half of the US population is deficient in this molecule and may need to take higher doses of coumadin than other people with a normal genetic makeup.

It is clear that under the right circumstances, unapparent genetic abnormalities of the heart or liver may cause sudden death. Just how often this occurs is not really known, but the number is neither inconsiderable nor insignificant. The ability to genetically screen for a broad range of disorders, even after death, has now opened new vistas to forensic pathologists.

Two areas come to mind immediately. The first is the clinical identification of relatives with mutations that predispose to sudden death. Lives will be saved that otherwise would not, and this possibility should be realized very soon. The only issue to be resolved is who will pay for the testing process. Fortunately, this is a concern for medical ethicists, not forensic pathologists. No medical examiner's office, at least in the United States, is in a position to fund this expensive technology. As a result, the decision will end up being made by elected authorities that, one hopes, will solicit the input of the physicians and medical ethicists. The other area where there will be immediate impact is drug-death investigation; it will soon be possible to accurately distinguish those who have died "from," a drug from those who have merely died "with" it.

The question is what to do in the interim, when it is clear to all that viable alterative explanations for some crimes and torts do exist, but it is impossible to rule them in or out. In some cases, it may simply make sense to save tissue for later testing (even if that means changing the laws of some countries!). In other instances, for example, a death-in-custody with a "nearly normal autopsy," where trivial levels of cocaine or MDMA are detected in postmortem blood samples, the only choice may be to use older, much more expensive, DNA technologies to get the answers. It will cost thousands of dollars that coroners will be loath to spend, and it will take months to do. But considering the overall expense of investigating and litigating a death-in-custody, the added expense does not seem very great.

The consequences in toxic tort litigation may be even greater. Suppose the decedent had been taking Vioxx™, or an antidepressant, and "therapeutic concentrations" were detected in the blood? Would the pathologist have been able to resist the temptation to attribute the death to Vioxx™ or the antidepressant? After all, both drugs are known to be associated with the occurrence of SCD, and the autopsy was "nearly normal".

In a recent review on the role of genetic analysis published in the journal Circulation, the official journal of the American Heart Association, Priori and Napolitano wrote that there are "compelling medical reasons to consider genetic testing an important component of (the) clinical management of affected individuals and their relatives." They also added "the lack of availability of genetic testing is related largely to the absence of appropriate reimbursement polices; thus it is important that

the scientific communities involved in researching these diseases feel committed to prioritize the collection of data that will facilitate transition of genetic analysis for cardiomyopathies and channelopathies into clinical practice. Partnering with stakeholders such as patients' associations, governments, insurance, and private companies involved in genotypic may be an effective approach to achieve these goals. It hardly needs stating, but all of us in the forensic community are also stakeholders.[46]

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